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Phycobionts of *Brigantiaea ferruginea* (Lichens)[1)]

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地衣類サビイボゴケ（*Brigantiaea ferruginea*）の共生藻[1)]

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Phycobionts of a crustose lichen, *Brigantiaea ferruginea* (Müll. Arg.) Kashiw. et Kurok., were isolated and their unialgal and axenic cultures were established. After careful examination, the alga was identified as *Dictyochloropsis reticulata* (Tsch.-Woess) Tsch.-Woess (Chlorophyceae, Chlorococcales). Morphological characters and asexual reproduction of the alga as cultured on an enriched, agarized medium under the standard condition of light and temperature are described. Literature concerned with the species is reviewed.

Brigantiaea ferruginea (Müll. Arg.) Kashiw. et Kurok. is a crustose lichen. It grows both on epiphytic mosses and tree trunks. No phycobiont of *B. ferruginea* has been reported yet. In this study, two strains of phycobionts were isolated from two specimens of this lichen. The phycobionts were studied taxonomically and the morphological features of them are described.

Materials and methods

Two living specimens of *Brigantiaea ferruginea* (Okamoto no. 1616 and Takeshita no. 608) were collected at Mt. Garyû, Hiroshima Prefecture, western Honshu, Japan. They were brought to the laboratory and used for isolation of the phycobionts. These lichen specimens are deposited in the herbarium of Hiroshima University (HIRO).

Unialgal strains of phycobionts were obtained by the following procedure. Small pieces of washed thalli of *Brigantiaea ferruginea* were ground between two glass slides. The suspensions of phycobiont cells mixed with mycobiont hyphae were spread on Bold's Basal Medium (BBM) agar plates as modified by Bischoff and Bold (1963), and cultured under the standard condition (20°C, 2000 lux, 12h light/12h dark cycles). After about one month, colonies of phycobionts were visible and these were transferred to BBM agar slants. Axenic strains were obtained by the spray method (Wiedeman et al. 1964). Morphological characters and life cycles of phycobionts cultured under the standard condition for two to three weeks were studied under the light microscope. Unialgal and axenic strains are deposited in the Botanical Institute, Faculty of Science, Hiroshima University (CCHU).

Results and discussion

In the thallus of *Brigantiaea ferruginea,* vegetative cells of phycobionts are unicellular and form an algal layer under the upper cortex. They are irregular in shape and the shape of chloroplasts is indistinct because of the influence of mycobiont hyphae (Figs. 2, 14). Autosporangia were rarely observed, whereas zoosporangia and aplanosporangia were never observed in the thalli.

In culture, mature vegetative cells of phycobionts in the log phase were spherical to subspherical, 10–15 μm in diameter (Figs. 6, 9, 15). In the stationary phase, old algal cells were about 18 μm in diameter and cell walls were up to 1 μm thick (Fig. 7). Chloroplasts were parietal and had no pyrenoid (Figs. 6, 15). Gelatinous matrices of algal cells were not observed.

Asexual reproduction occurred through production of autospores, zoospores and aplanospores. Autosporangia were often observed in log phase cultures. Each sporangium produced 2–16 autospores. Zoosporangia were often observed in log phase cultures but zoospores were observed only during a restricted period (about one hour) in early morning. Zoospores showed a characteristic shape, and they were 3.5 μm in width, 6 μm in length, and had two flagella of equal length which emerged apart from each other at the anterior end of the zoospores (Figs. 10, 11). Zoospores moved rapidly for a while, and then they lost mobility and became rounded (Fig. 11). An observable stigma was not observed. Sexual reproduction was not observed in this study.

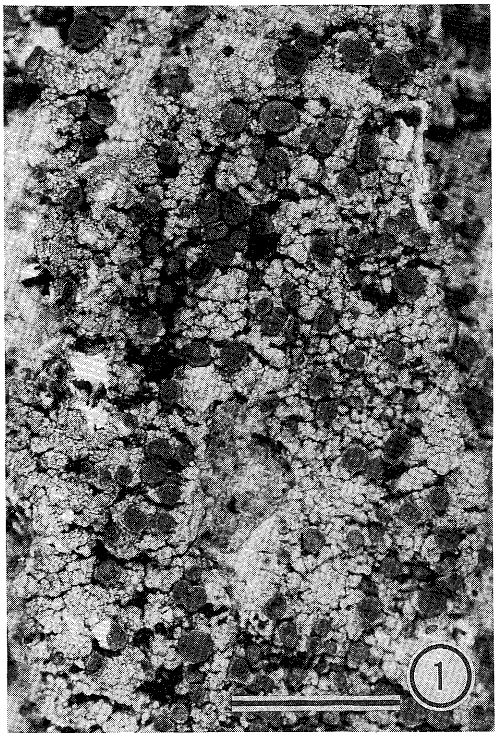
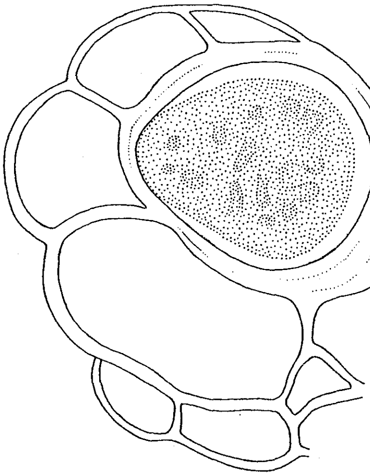


Fig. 1. *Brigantiaea ferruginea,* crustaceous thallus with apothecia. Scale bar indicates 5 mm.

The characteristics of isolated phycobionts agreed well with those of *Dictyochloropsis reticulata* (Tsch.-Woess) Tsch.-Woess (Chlorophyceae, Chlorococcales). This species was first reported by Tschermak-Woess (1951) as *Myrmecia reticulata* isolated from the lichen *Catillaria chalybeia.* Later, Tschermak-Woess (1984) revised this species and transferred it to *Dictyochloropsis* because of the morphological differences of chloroplasts and zoospores. Tschermak-Woess (1978) reported *D. reticulata* as the phycobionts

Figs. 2–13. *Dictyochloropsis reticulata* (Tsch.-Woess) Tsch.-Woess. 2. Vegetative cell in living thallus of *Brigantiaea ferruginea.* 3–5. Young vegetative cells in axenic cultures. 6. Median optical section of the mature vegetative cell in the culture. 7. Old vegetative cell in the culture. 8. Autosporangium with 8 spores in the culture. 9. Surface view of the same cell of Fig. 6. 10. Zoospore with two flagella of equal length. Note apart basis of flagella. 11. Zoospore settled and rounded. 12. Zoosporangium in the culture. 13. Aplanosporangium in the culture. Scale bar indicates 10 μm.



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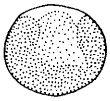

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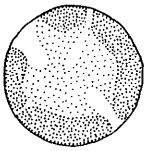

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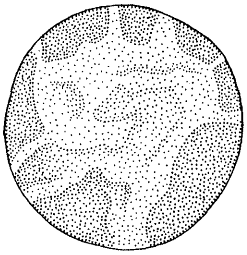

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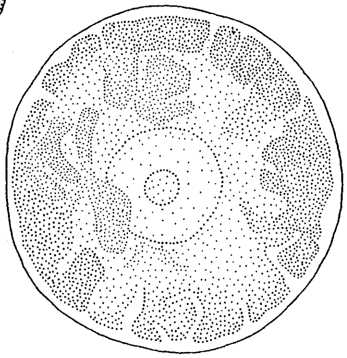

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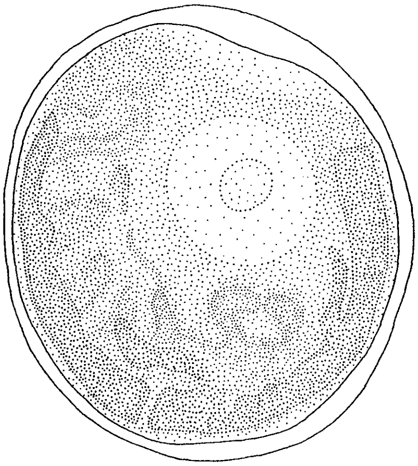

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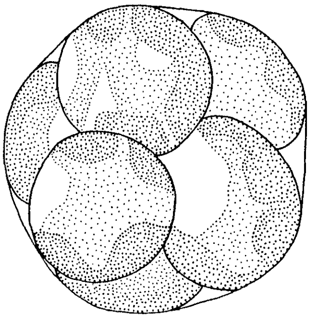

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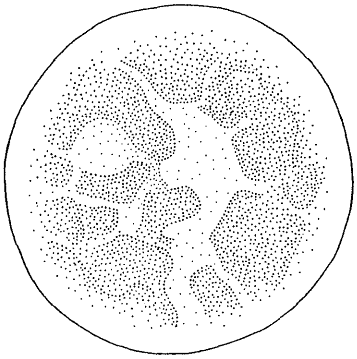

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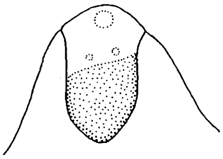

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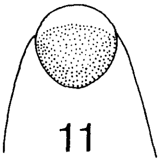

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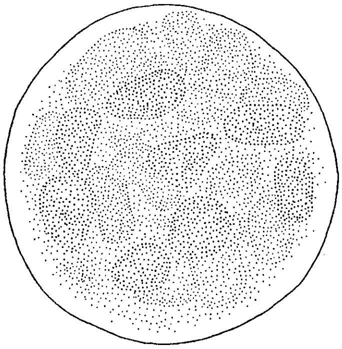

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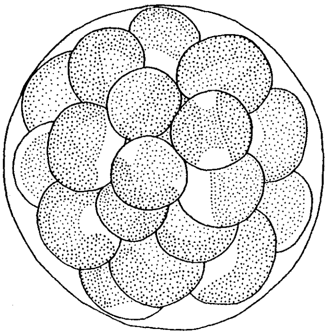

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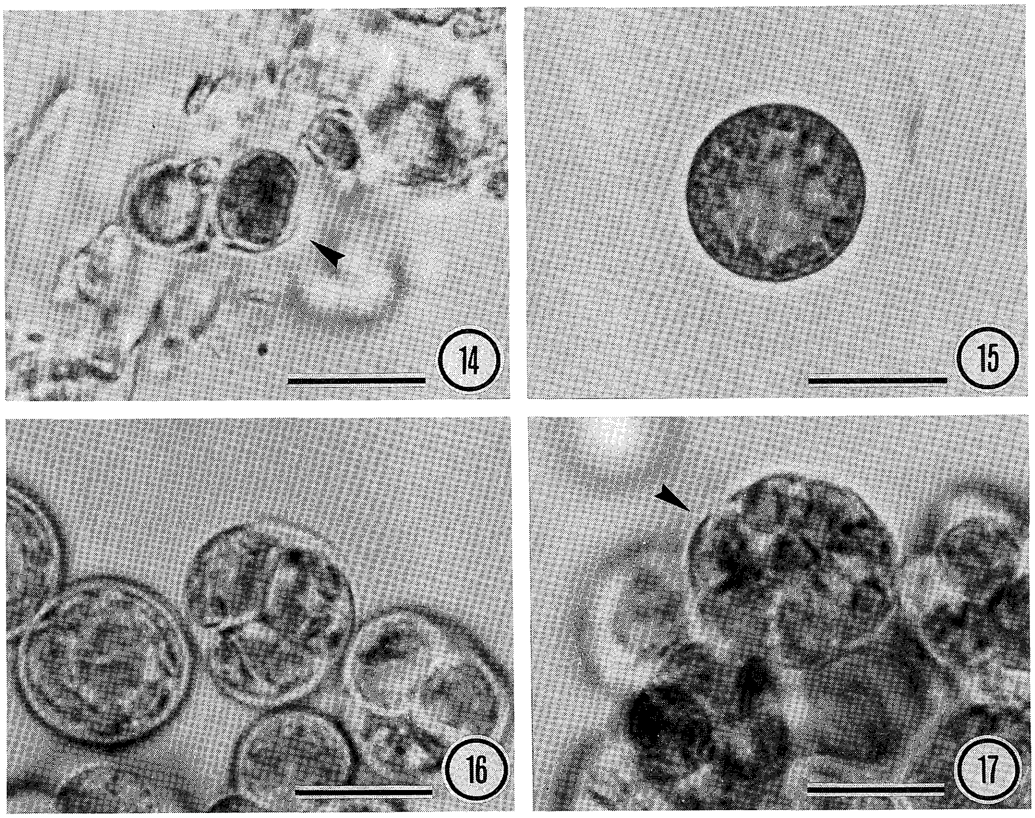

Figs. 14–17. *Dictyochloropsis reticulata* (Tsch.-Woess) Tsch.-Woess. 14. Vegetative cell in living thallus of *Brigantiaea ferruginea* (arrowhead). 15. Vegetative cell in axenic culture. 16. Autosporangia with 4 spores. 17. Autosporangium with 16 spores (arrowhead). Scale bars indicate 10 μm.

of six species of other lichen genera and also found it as a free-living alga. In Japan, *D. reticulata* was reported as a corticolous alga (Nakano et al. 1991). The present study is the first report of the phycobionts of *Brigantiaea ferruginea.*

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要　旨

地衣類サビイボゴケ（*Brigantiaea ferruginea*）は樹木着生の蘚類上に生育する痂状地衣類で，現在までこの地衣類の共生藻に関する報告はない．今回，広島県山県郡芸北町臥竜山で本種の標本2点を得たので，共生藻を分離・培養し，分類学的検討を行なった．その結果，共生藻として緑藻類の*Dictyochloropsis reticulata*が認められた．本藻の細胞は，地衣体内では球形や長円形など，かなり不規則な形をしているが，培養後は直径15μm程度の球形～亜球形となる．葉緑体は側壁性で，網目状をしており，ピレノイドを持たない．無性生殖は，地衣体内では自生胞子形成のみが観察された．培養株では主に自生胞子形成を行なうが，遊走子や不動胞子の形成もまれに観察された．有性生殖は観察されなかった．*D. reticulata*は，日本では樹皮着生藻類としての報告はあるが，地衣類の共生藻としては今回が初めての報告である．

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